

AMENDMENTS TO THE CLAIMS

1. **(Currently Amended)** ~~[[M]]~~ A method for determining an absolute concentration of one or more mRNA molecules in a sample, comprising mRNA quantities by means of DNA microarrays with the following steps:
 - a. providing a DNA microarray comprising a plurality of immobilized probes and including one or more dilution series of control spots;
 - b. ~~providing at least one or more dilution series of control spots on the microarray~~ hybridizing the immobilized probes with the sample or cDNA made from the sample;
 - c. hybridizing the immobilized probes with a corresponding control DNA of known concentration;
 - d. deriving reference data from ~~the hybridization step c;~~ and
 - e. using said reference data to calculate absolute mRNA concentrations in said sample one or more samples used.
2. **(Currently Amended)** ~~[[M]]~~ The method according to claim 1, further comprising the step of providing a universal tag at each of the immobilized probes on the microarray to be used for quantification.
3. **(Currently Amended)** ~~[[M]]~~ The method according to claim 1 or 2, wherein [[a]] the DNA microarray is a cDNA microarray, and/or the control DNA is control cDNA, or both.
4. **(Currently Amended)** ~~[[M]]~~ The method according to claim 1 or 2 any of the preceding claims, wherein the reference data comprise a model curve which is fitted or adapted to the obtained signals obtained from the [[for]] control spots.
5. **(Currently Amended)** ~~[[M]]~~ The method according to claim 1 or 2 any of the preceding claims, wherein cDNA probes templates for the genes that are to be probed for are first amplified by large-scale multiplex polymerase chain reaction (PCR) to obtain amplified fragments prior to immobilization on the DNA microarray.

6. **(Currently Amended)** [[M]] The method according to claim 5, wherein said amplified fragments are then transferred to the microarray by means of robotic ~~roboting~~ devices which are able to deliver nanoliter quantities with a spatial precision of better than 100 μm .

7. **(Currently Amended)** [[M]] The method according to claim 1 or 2 ~~any one of the preceding claims~~, wherein the reference data of step d ~~obtained from the hybridization, e.g., the read-out signal intensities from the dilution series spots~~, are taken as a basis for calculating [[the]] parameters of a model function by means of non-linear least-squares fitting.

8. **(Currently Amended)** [[M]] The method according to claim 7, wherein for the model function the following function is used:

$$\hat{I} = \frac{KI_0 e^{rc_p}}{K + I_0(e^{rc_p} - 1)}$$

wherein the function has a modeled signal intensity, a probe (or DNA spot) concentration, an asymptotic signal intensity for $c_p \rightarrow \infty$ and an asymptotic signal intensity for $c_p \rightarrow 0$, and where \hat{I} refers to the modeled signal intensity, c_p refers to the probe (or DNA spot) concentration, K represents the asymptotic signal intensity for $c_p \rightarrow \infty$, I_0 is the asymptotic signal intensity for $c_p \rightarrow 0$, and r is a shape parameter.

9. **(Currently Amended)** [[M]] The method according to claim 7 ~~any one of claims 4-8~~, wherein fitting ~~of~~ the reference data is done by gradient optimization procedures.

10. **(Currently Amended)** [[M]] The method according to claim 9, wherein Newton-Raphson fitting is used for non-linear fitting, ~~the Newton-Raphson method is used~~.

11. **(Currently Amended)** [[M]] The method according to claim 1 or 2 ~~any one of the preceding claims~~, wherein a critical probe function is used to determine [[the]] a set of spots whose values

need correction for ~~[[the]]~~ influence of spot DNA concentration, wherein a ~~[[the]]~~ critical probe concentration function is ~~[[being]]~~ defined by

$$c_{crit} = \frac{1}{r} \left[\ln \frac{17(K - I_0)}{3I_0} \right].$$

12. **(Currently Amended)** ~~[[C]]~~ A computer program product comprising program code means stored on a computer readable medium for performing ~~[[the]]~~ a computable part of the method of claim 7 ~~at least one of the preceding claims~~ when said program product is run on a computer.

13. **(Currently Amended)** ~~[[S]]~~ A system, ~~particularly~~ for performing the method of claim 1 or 2 ~~at least one of claims 1-11~~, comprising:

- a. a microarray containing at least one or more dilution series of control spots;
- b. means for hybridizing with a complementary control DNA of known concentration;
- c. means for deriving reference data from ~~said hybridization step b~~, and
- d. means for making use of said reference data for calculating absolute mRNA concentrations ~~in one or more samples used~~.

14. **(Currently Amended)** ~~[[S]]~~ A system according to claim 13, further comprising means for providing a universal tag at each of the immobilized probes on the microarray to be used for quantification.

15. **(Currently Amended)** ~~[[Use of a]]~~ A method for determining an absolute quantity of one or more mRNA molecules in a sample, comprising performing the method of claim 7 according to at least one of claims 1-11 using a computer to execute a computer program that performs calculations that carry out at least part of the method. product according to claim 12 and/or a system according to at least one of claims 13 or 14 for determining absolute mRNA quantities on cDNA microarrays.

16. **(Cancelled)**

17. (New) A method for determining an absolute quantity of one or more mRNA molecules in a sample, comprising performing the method of claim 7 using a system comprising:
- a. a microarray containing at least one or more dilution series of control spots;
 - b. means for hybridizing with a complementary control DNA of known concentration;
 - c. means for deriving reference data from step b, and
 - d. means for making use of said reference data for calculating absolute mRNA concentrations in one or more samples used.
18. (New) A method for determining an absolute concentration of one or more nucleic acid molecules in a sample, comprising:
- a. providing a DNA microarray having a plurality of immobilized probes and one or more dilution series of control spots;
 - b. hybridizing the immobilized probes with the sample or cDNA made from the sample;
 - c. hybridizing the immobilized probes with a corresponding control nucleic acid of known concentration;
 - d. deriving reference data from step c; and
 - e. using said reference data to calculate absolute nucleic acid concentrations in said sample.